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Coupling of microbial nitrogen transformations and climate in sclerophyll forest soils from the Mediterranean Region of central Chile

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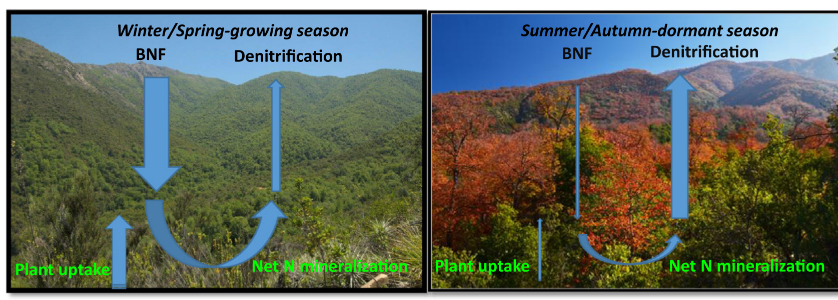
HIGHLIGHTS

- Microbial N transformations evidence a strong seasonal pattern.
- Diazotrophic activity and net N mineralization decrease during summer drought.
- Denitrification increase during summer drought.
- Water addition in field experiments increase diazotrophic activity.
- Predicted increase in summer drought period may lead to a depletion of soil N.

GRAPHICAL ABSTRACT



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ABSTRACT

The Mediterranean region of central Chile is experiencing extensive "mega-droughts" with detrimental effects for the environment and economy of the region. In the northern hemisphere, nitrogen (N) limitation of Mediterranean ecosystems has been explained by the decoupling between N inputs and plant uptake during the dormant season. In central Chile, soils have often been considered N-rich in comparison to other Mediterranean ecosystems of the world, yet the impacts of expected intensification of seasonal drought remain unknown. In this work, we seek to disentangle patterns of microbial N transformations and their seasonal coupling with climate in the Chilean sclerophyll forest-type. We aim to assess how water limitation affects microbial N transformations, thus addressing the impact of ongoing regional climate trends on soil N status. We studied four stands of the sclerophyll forest-type in Chile. Field measurements in surface soils showed a 67% decline of free-living diazotrophic activity (DA) and 59% decrease of net N mineralization rates during the summer rainless and dormant season, accompanied by a stimulation of in-situ denitrification rates to values 70% higher than in wetter winter. Higher rates of both free-living DA and net N mineralization found during spring, provided evidence for strong coupling of these two processes during the growing season. Overall, the experimental addition of water in the field to litter samples almost doubled DA but had no effect on denitrification rates. We conclude that coupling of microbial mediated soil N transformations during the wetter growing season explains the N enrichment of sclerophyll forest soils. Expected increases in the length and intensity of the dry period, according to climate change models, reflected in the current mega-droughts may drastically reduce biological N fixation and net N mineralization, increasing at the same time denitrification rates, thereby potentially reducing long-term soil N capital.

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1. Introduction

Mediterranean-type ecosystems are currently subjected to increasing drought stress due to more intense heat and seasonal droughts, a phenomenon that is common to all five regions of the world where they occur (IPCC, 2013). Over the past three decades, central Chilean climate has been marked by the occurrence of an extensive "mega-drought", which is driven to large extent by anthropogenic climatic forcing (Boiser et al., 2016). Such "mega-drought" has important consequences for the storage and provision of water and nutrients in managed and natural ecosystems, for the frequency of both human-set as well as wildfires affecting ecosystems and human property, and for the long-term survival of tree populations in the region (CR2, 2015).

Element cycles such as nitrogen (N) are strongly driven by biotic and climatic variables that are subjected to strong seasonal and supra-annual fluctuations in Mediterranean-type ecosystems. These ecosystems are characterized by limited N availability because of reduced moisture levels during the extended dry summer period and the poor chemical quality of senescent organic matter, which slows down recycling in sclerophyll woodland soils by influencing decomposition and mineralization processes. Accordingly, water and nutrient availability, N availability in particular, are the most frequent limiting factors for primary productivity in Mediterranean-type ecosystems (Sardans and Peñuelas, 2013; Delgado-Baquerizo et al., 2016).

The main process supplying "new" N to remote, less polluted ecosystems, such as Artic, Sub-Artic, southern temperate, and Sub-Antarctic forests, is biological nitrogen fixation (BNF) (DeLuca et al., 2002; Pérez et al., 2003; Rousk et al., 2015, 2017; Pérez et al., 2017). This process is ordinarily carried out by diazotrophic bacteria, either free-living or symbiotic, which are endowed with the nitrogenase enzyme complex that enables them to transform atmospheric molecular N_2 directly to ammonia. Heterotrophic and autotrophic soil organisms, on the other hand, decompose fixed organic N, which becomes dissolved in the soil solution and mineralized into inorganic N forms, such as ammonium and nitrate that can be readily taken up by microbes and plants, thereby being retained within the ecosystem (Chapin et al., 2002). However, microbial-mediated dissimilatory nitrate reducing processes can cause N losses from the ecosystem via denitrification, which is carried out by denitrifying bacteria that reduce nitrate to nitrous oxide (N_2O), a potent greenhouse gas (Chapin et al., 2002). All of these oxidizing-reducing series of processes are mediated by a wide array of microbial enzymes that are strongly regulated by multiple feedbacks related to substrate availability, as well as by local changes in air temperature and soil moisture levels. Consequently, key microbial-mediated N transformations, such as BNF, net N mineralization, and denitrification processes that are essential for maintaining soil N pools, can be altered under current global change (Vitousek, 1994).

Examples of climatic alterations are frequently reported for Mediterranean-type regions. In fact, a global comparison of arid and semiarid regions shows that an observed increase in the aridity index has caused a drop in the pools of total N and C, and N mineralization rates in soils (Delgado-Baquerizo et al., 2013, 2016). A recent global meta-analysis reported enhanced gaseous N losses from soils, as nitric oxide emissions via denitrification, in response to experimental drought (Liu et al., 2017). Moreover, temporal variability of N pulses in Mediterranean ecosystems could mean even greater vulnerability to global change (Sardans and Peñuelas, 2013). In highly seasonal, arid and semi-arid environments, under high industrial N deposition, as in the northern hemisphere, nutrients are supplied in pulses following rain events, which in Mediterranean regions are restricted to the winter months (Fenn et al., 2003). In the North American chaparral, it is well documented that available soil N increases after the first winter rain, causing an abrupt pulse of hydrological N losses, mainly because of the decoupling of N inputs and plant uptake during the dormant season (Vourlitis et al., 2009). As a consequence, despite the high dry N deposition occurring during the dry season, the decoupling of rainfall and N inputs maintains N limitation in North American Mediterranean-type ecosystems (Ochoa-Hueso et al., 2014; Homyak et al., 2014). In contrast, soils from the Mediterranean region in central Chile are often considered N-rich, when compared with other Mediterranean-climate regions of the world, and with the California chaparral in particular (Miller, 1981; Rundel, 1982; Shaver, 1983; Stock and Verboom, 2012; Sardans and Peñuelas, 2013).

The main goal of this study is to disentangle the patterns of the major microbial N transformations, i.e., free-living N fixation, N mineralization, and denitrification, and their correspondence with the Mediterranean-climate seasonal contrasts in the sclerophyll forest-type from Central Chile. We will experimentally assess how water supply regulates BNF and denitrification processes.

We asked the following three questions about soil N transformations:

1. How do microbial N transformations differ among seasons in the Mediterranean-type ecosystem of central Chile? We expect to find evidence for a pronounced decline of BNF, N mineralization and denitrification during the dry summer and dormant season.
2. Does experimental water addition to sclerophyll forest soils in the field increase the rates of BNF and denitrification? We expect to find evidence for water limitation of both processes.
3. Is the coupling of microbial N transformations during the wetter growing season a key factor explaining the reported higher N status in Chilean Mediterranean-climate soils?

Answering these questions will reveal whether the coupling of microbial N transformations and seasonal climate could determine the amount of soil N that would become available for plant uptake during the growing season, and eventually determine the retention or loss of soil N capital. Moreover, understanding the responses of both BNF and denitrification processes to experimentally enhanced water supply, and to the seasonal variation in the patterns of net N mineralization, will be useful information to predict the vulnerability of Mediterranean-type ecosystems to increasing summer drought, as such tendency predicted by climate change models for central Chile.

2. Materials and methods

2.1. Study sites

We studied four stands of the sclerophyll forest-type, located on the Coastal Range at the Nature Reserve "Roblería del Cobre de Loncha" (RCL), where the co-dominant trees in the canopy were evergreen sclerophyllous trees and deciduous *Nothofagus obliqua* (southern beech). The Reserve is located 80 km southwest of the city of Santiago (Fig. 1) in the Mediterranean region of central Chile. Mean annual precipitation is 454 mm with a mean annual temperature of 16.6 °C. The majority of the annual precipitation (63%) falls during the austral winter, from June to August, with only 0.66% of it falling during the austral summer months, December–March (https://es.climate-data.org). Field campaigns were conducted each season (summer, fall, winter and spring) from September 2015 (late winter) to March 2016 (early autumn). This was a year with low annual precipitation, reaching only 170 mm, according to records from the closest meteorological station to the study site (Santiago). Seasonally, 78% of the rain fell during the winter months (June–August), with complete lack of rain from early summer, December 2015, to early autumn, March 2016 (www.meteochile.cl). A decline of about 30% in mean annual precipitation, has been occurring since 2010 in central Chile and climatologists have called this climatic anomaly a "mega-drought" (Boiser et al., 2016; CR2, 2015).

Geological substrate for all sites are primarily granitic/granodioritic mixed with volcanic sediments. Soil type belongs to typical Xerochrepts developed over colluvium material (FAO-UNESCO, 1987). The Nature

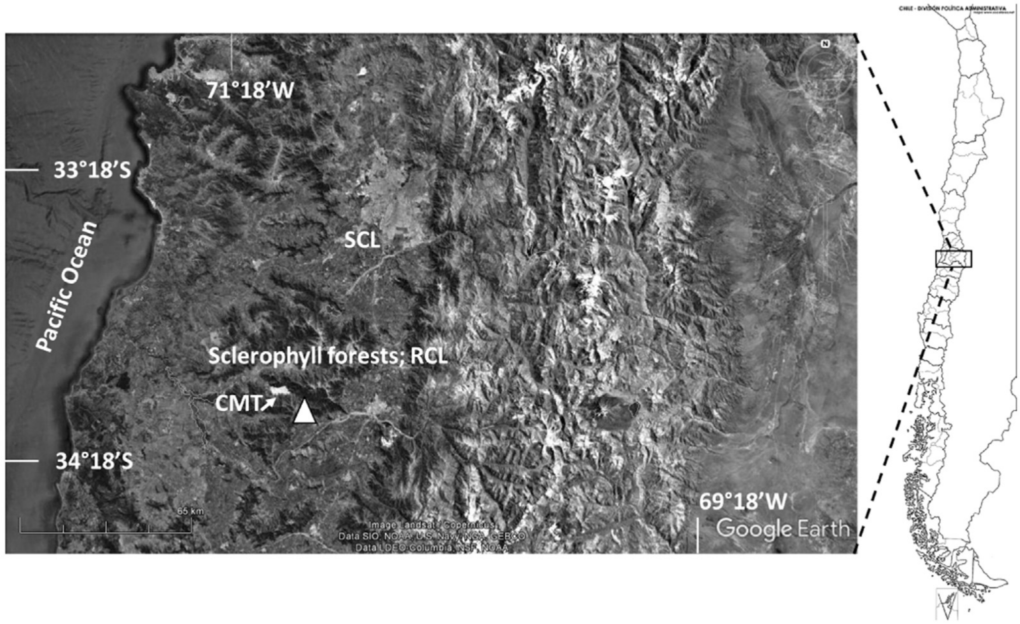

Fig. 1. Map of study area in the Mediterranean-climate Region of central Chile. The Nature Reserve "Roblería de los Cobres de Loncha" (RCL) found on the Coastal Range, where four stands of the sclerophyll forest-type were studied. CMT: copper mine tailing, SCL: Santiago city.

Reserve presents a less intense history of anthropogenic disturbances than the surrounding landscape, which was subjected to high pressure from agriculture, cattle grazing, firewood and litter extraction, anthropogenic fire, and housing developments, leading to extensive replacement of the original vegetation (Armesto et al., 2007).

Within the Nature Reserve we selected four forest stands for sampling (Table 1): Stand (1) is a mixed sclerophyll forest located at an elevation of 1205 m (Table 1), where tree and shrub species such as *Peumus boldus*, *Cryptocarya alba,* and *Lithrea caustica* among others, constitute the main canopy, with scattered taller individuals of the deciduous *Nothofagus obliqua*. Stand (2) is a pure sclerophyll forest, with the same canopy, but without *Nothofagus* trees (Table 1). Stand (3) is a mixed deciduous *Nothofagus* forest at lower elevation (635 m, Table 1), where dominant individuals of the deciduous *Nothofagus obliqua* are mixed in with sclerophyllous and mesic tree species, such as *Drimys winteri* and *Sophora macrocarpa*. Finally, Stand (4) is a sclerophyllous forest located within the Reserve, and in the proximity where a copper mine tailing is deposited (SCMT, hereafter). Since 1987, copper mine tailings are being deposited over an intensively grazed valley bottom (Fig. 1). As a legal compensation for the environmental impact, the owner of the mine (National Copper Corporation of Chile, Codelco) donated in 1996, 5870 ha of forested land to the Corporación Nacional Forestal (Chilean Forest and Parks Service), which is today the core of the Nature Reserve.

The estimated amount of N in wet deposition over the study region is <1 kg N ha^{-1} y^{-1} (Vet et al., 2014), which coincides with our empirical estimates of 0.749 kg N ha^{-1} y^{-1} for a comparable study site in a nearby location of central Chile (Pérez et al., unpublished data). Dry deposition was estimated in ca. 1–2 kg N ha^{-1} y^{-1} (Vet et al., 2014). Therefore, inputs of reactive N via bulk deposition to the study area can be characterized as low.

2.2. Microbial nitrogen transformations

Rates of diazotrophic activity (DA) were estimated by the acetylene reduction assay (Myrold et al., 1999) for the main N fixer community-types observed in the field: free-living diazotrophs from the compound layer of litter (Oi-soil horizon) plus the top 10 cm of mineral soil (Ah-soil horizon). To estimate diazotrophic activity samples were incubated in the field, each season, for up to two days. Samples of litter plus mineral soil (in 100 cm^3 soil cores) were incubated (at field temperature and equivalent light levels) inside hermetically closed 500 ml jars,

Table 1
Dominant and co-dominant tree species, altitude, and geographic coordinates for the four sclerophyll forest stands studied in the Nature Reserve "Roblería del Cobre de Loncha" (RCL) in the Mediterranean-climate region of central Chile.

Study sites	Dominant/co-dominant tree species	Elevation (m.a.s.l)	Latitude/Longitude
Mixed –sclerophyll forest, with evergreen canopy dominants and scattered *Nothofagus*	*Lithraea caustica* *Peumus boldus Cryptocarya alba* *Azara serrata* *Citronella mucronata Nothofagus obliqua*	1205	34°10′8.52″S 70°57′53.94″W
Pure sclerophyll forest	*Lithraea caustica* *Peumus boldus Cryptocarya alba* *Quillaja saponaria*	719	34° 8′47.29″S 70°58′5.10″W
Mixed-deciduous, with deciduous *Nothofagus* as canopy dominant	*Nothofagus obliqua Sophora macrocarpa Cryptocarya alba* *Drimys winteri*	635	34° 9′4.19″S 70°58′41.80″W
Pure Sclerophyll disturbed by copper mine tailing (SCMT)	*Lithraea caustica* *Peumus boldus* *Cryptocarya alba* *Quillaja saponaria*	217	34°7′24.92″S 71° 4′24.64″W

containing a mixture of air and pure acetylene (10% v/v). Six samples were incubated after 10 ml deionized water addition and other six control samples remained under field conditions regarding water content. Water additions increased the substrate water content by ca. 50%. An additional sample of each N fixer community-type was incubated without acetylene as control. One gas sample per jar was taken every day and analyzed for ethylene production using a Schimadzu gas chromatograph, equipped with a Porapack column and a FID detector. Ethylene concentration was determined from a calibration curve by diluting 100 ppm ethylene balance in nitrogen of Scotty analyzed gases. Acetylene reduction rates to ethylene (a measure of diazotrophic activity) was estimated from the slope of the lineal fit of ethylene production during incubation in the corresponding headspace and referred to dry weight of substrate. Seasonal rates of BNF fixation were estimated assuming a theoretical conversion factor of 1/3 of the acetylene reduction activity, multiplied by the respective biomass of the N fixer community type. Annual estimates were obtained by adding seasonal values of BNF. Litter biomass was estimated by collecting fine litter within a 25*25 cm frame, with 12 replicates per site, oven dried and weighted in the laboratory. Bulk density of mineral soil was estimated from the same soil cores used for the in-situ experiments.

Net N mineralization rates were estimated in the field for each site and season using sieved (2 mm mesh size) surface soils (0–10 cm) by the "buried bag method" (Eno, 1960). These soil samples exclude roots, but include soil N that could be immobilized by soil microbes. Soils at time zero and after one month of field incubations were extracted in a 0.021 mol L^{-1} $KAl(SO_4)_2$ solution (1:4) and ammonium and nitrate concentrations were determined by fractionated steam distillation (Pérez et al., 1998). Available N (N_a) was calculated as the sum of ammonium-N plus nitrate-N. Rates of net N mineralization were obtained by subtracting total available N estimated after incubation from the N available at time zero, relative to incubated soil dry mass.

In situ denitrification rates for each site and season were estimated by the acetylene inhibition (from N_2O to N_2) assay (Groffman et al., 1999) using intact soil cores (100 cm^3) placed inside 500 ml hermetic glass jars and incubated for 6 h under a 10% v/v acetylene atmosphere. Six soil cores were incubated under field water content and six with the addition of 10 ml deionized water. Gas samples were obtained after 2 and 6 h of incubation. The N_2O concentration accumulated in the gas samples was determined using a Shimadzu gas chromatograph equipped with a Porapack column Q 80/100 and electron capture detector. Calibration curves were prepared by diluting a 1-ppm N_2O balance of nitrogen Scotty analyzed gases. Denitrification rates were estimated from N_2O-N concentration differences between values 2 and 6 h after incubation, and referred to an area basis. Considering that acetylene inhibits ammonium oxidation to nitrate, denitrification rates estimated here were assumed to use nitrate already present in the soil core (Walter et al., 1978).

2.3. Chemical analysis of soil, litter and fresh leaves

In each of the four study sites, we randomly placed six sampling points, separated by 10 m from each other. Each season, soil samples of surface soils (first 10 cm of mineral soil) were taken with a shovel from beneath the litter layer. In the laboratory, samples were sieved using a 2-mm mesh size before analysis. Additionally, hand reachable fresh and shaded leaves were taken from three individuals of each of the main evergreen sclerophyll species, *Lithraea caustica* and *Peumus boldus*, which are common to both types of sclerophyll stands studied; the pure sclerophyll forest without *Nothofagus*, and the sclerophyll stand found close to the copper mine tailings in the study site. Soils, litter (from the control samples in the incubation jars) and green leaves were dried at 70 °C and ground mechanically for analysis of total N and C using flash combustion in a NA2500 Carlo Erba Element Analyzer. Total P present in ground litter and soil material was extracted with concentrated sulfuric acid, in a water peroxide solution using a Hach Digesdahl Digestor and determined by colorimetry with the molybdenum-blue method (Steubing and Fangmeier, 1992).

Plant available P was extracted from sieved fresh soils through lactation by the CAL (Calcium-Acetate-Lactate) method and determined colorimetrically by the molybdenum blue method (Steubing and Fangmeier, 1992). Exchangeable base cations, calcium, sodium, potassium and magnesium, in soil were recovered from fresh soils in a 1 M ammonium acetate solution (1:10) and determined using a Perkin Elmer 2380 AAS (Robertson et al., 1999). Water content of soil and litter samples was determined gravimetrically. Soil reaction was determined with a pH electrode in a 1:2, soil to water suspension. All laboratory analyses were performed at the Biogeochemistry Laboratory, Pontificia Universidad Católica de Chile, Santiago.

2.4. Statistical analyses

To assess significant differences in chemistry of mineral soil and litter, and soil microbial N transformations among sites, a mean annual value was calculated from the seasonal values for each sampling point in each site. After testing for variance homogeneity, either one-way ANOVAs and a-posteriori Tukey's tests, or Kruskall-Wallis and multiple comparisons, were applied. Owing to the high frequency of null values, rates of in situ microbial N transformations (diazotrophic activity, net N mineralization, and denitrification) were box-cox transformed. To assess seasonality and site-specific effects, we compared DA and denitrification of control vs. water addition treatments, using two factor ANOVAs for repeated measures (season effect) on the box-cox transformed data. To assess the seasonal variation in the N content of fresh leaves two factors (species, site) ANOVAs for repeated measures (season effect) was applied on log-transformed data from two sclerophyll stands and for the two most common species mentioned above. To evaluate the effects of season and site on litter and soil water content, net N-mineralization rate and soil nitrate content, one-way ANOVAs for repeated measures (season effect) were applied. Analyses were performed with the statistical program Systat 7.1 for Windows.

3. Results

3.1. Seasonal variation of soil and litter water content

There were significant season and site effects on water contents of surface soils and litter of the sclerophyll stands (Table 2). Water contents of the litter layer were reduced by up to 32% and in surface soil by up to 80% in both the austral summer and autumn compared to winter and spring (Fig. 2a, b). In winter, the mixed deciduous stand had higher water content than all other sclerophyllous stands studied (Fig. 2a). During spring, litter of the mixed sclerophyll stand presented significantly higher water content than all other sclerophyll stands studied (Fig. 2a). During autumn the litter layer of the SCMT presented significantly lower water content than the mixed sclerophyll stand. Surface soil of the SCMT presented significantly lower water content than both the mixed sclerophyll stand during spring and the mixed deciduous stand in winter (Fig. 2b).

Table 2
One-way ANOVAs for repeated measures (season) for water content in the litter layer and surface soils of the four sclerophyll stands.

	Litter layer			Surface mineral soil		
Effect	df	F	P	df	F	P
Site	3	22.5	<0.001	3	8.2	0.001
Season	3	31.7	<0.001	3	58.8	<0.001
Season* Site	9	12.7	<0.001	9	6.7	<0.001

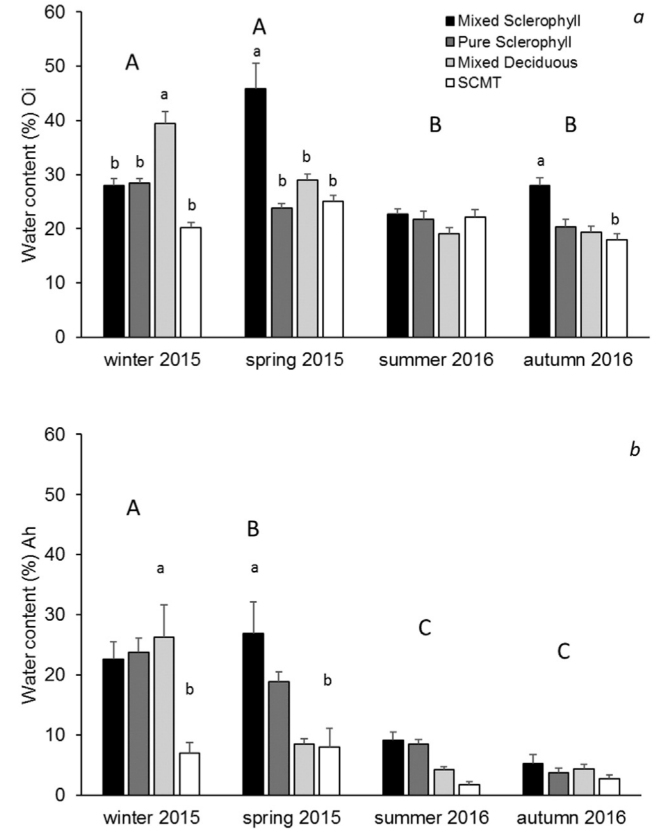

Fig. 2. Seasonal water content (%) in the litter layer (a) and surface soils (b) in the sclerophyll forest-type: Mixed sclerophyll, Pure sclerophyll, Mixed deciduous, sclerophyll stand near the copper mine tailing (SCMT). Columns represent mean values for each forest stand (n = 6 ± SE).Different capital letters indicate significant differences among seasons, according to Tukey-test. Different lowercase letters indicate significant differences among sites, according to Tukey-test.

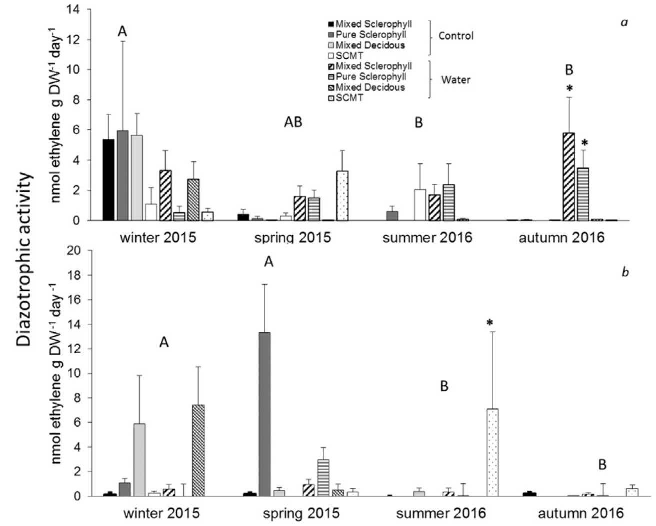

Fig. 3. Field-measured diazotrophic activity in the litter layer (a) and in surface mineral soils (b) of the sclerophyll forest-type: Mixed sclerophyll, Pure sclerophyll, Mixed deciduous, sclerophyll stand near the copper mine tailing (SCMT). Measurements were obtained from control (plain columns) and water added (stripped columns) treatments during the four seasons, from winter 2015 to autumn 2016. Columns represent mean values for each forest stand ($n = 6 \pm$ SE). Different capital letters indicate significant differences among seasons, according to Tukey-test. Asterisks indicate significant differences between control and water added treatment, according to Tukey-test.

3.2. Diazotrophic activity

Free-living DA in the litter layer of the sclerophyll stands varied greatly depending on the season, treatment and site (Table 3). DA was significantly lower during the drier summer (56% less) and autumn (41% less) than in the wetter winter season. On the other hand, across sites and seasons, water addition significantly stimulated DA up to 46% (Table 3; Fig. 3a). However, significant season*site*treatment interactive effects indicate that water addition significantly stimulated DA only during autumn. The increment was 94% in the mixed sclerophyll and 85% in the pure sclerophyll stands (Fig. 3a). Moreover, DA was 46% higher in litter from the pure sclerophyll stand and in 53% higher in the mixed sclerophyll stands than DA measured in both SCMT and the mixed deciduous stand (Fig. 3a). Fee-living DA on surface soils of the sclerophyll stands presented significant seasonal effect (Table 3), with lower values in the dry summer and autumn (67% less) than in the wetter winter and spring (Fig. 3b). There were no water addition or site effects on DA (Table 3). However, the significant site*treatment*season effect (Table 3) indicates that water addition completely stimulated DA, but only during summer in soils of the SCMT stand (Fig. 3b).

Estimated mean annual BNF rate in the litter layer was significantly higher in the mixed sclerophyll stand than in the SCTM stand (Table 1S). In the mineral surface soil, BNF was higher in the pure sclerophyll stand than both the mixed sclerophyll and the SCMT stands (Table 1S).

Table 3
Two-factor ANOVAs for repeated measures (season) of free-living diazotrophic activity in the litter and surface soils of the four sclerophyll stands, considering a water addition treatment.

	Litter layer			Surface mineral soil		
Effect	df	F	P	df	F	P
Site	3	10.2	<0.001	3	1.63	0.198
Treatment	1	26.88	<0.001	1	0.4	0.531
Site*treatment	3	5.82	0.002	3	6.14	0.002
Season	3	5.18	0.002	3	12.41	<0.001
Season*site	9	5.79	<0.001	9	11.48	<0.001
Season*treatment	3	6.87	<0.001	3	2.18	0.094
Season*treatment* site	9	2.16	0.03	9	4.46	<0.001

3.3. Net N mineralization

Net N mineralization rates in the surface mineral soil of these sclerophyll stands showed significant seasonal and site effects (Table 4). Higher rates of N mineralization (up to 59%) were measured in spring than in summer and autumn, and rates were 53% higher in winter than in summer (Fig. 4). Net N mineralization rates also varied greatly among sites, with 60% higher rates in the pure sclerophyll stand compared to all other stands. Significant interaction effects (Table 4) indicate that pure sclerophyll stand presented significantly higher rates in summer (76–100%) than in all other stands. During autumn net N mineralization in the pure sclerophyll were 77% higher than in the mixed deciduous stand (Fig. 4). During winter the mixed deciduous stand presented net N mineralization rates that were 63% higher than in the mixed sclerophyll stand (Fig. 4).

Likewise, nitrate concentrations in the surface mineral soils of all stands presented significant seasonal and site effects (Table 2S). Higher

Table 4
One-way ANOVAs repeated measures (season) for net N mineralization rates in surface mineral soils of the four sclerophyll stands.

Effect	SS	df	F	P
Site	13.19	3	20.8	<0.001
Season	8.69	3	7.69	<0.001
Season*site	9.45	9	2.79	0.008

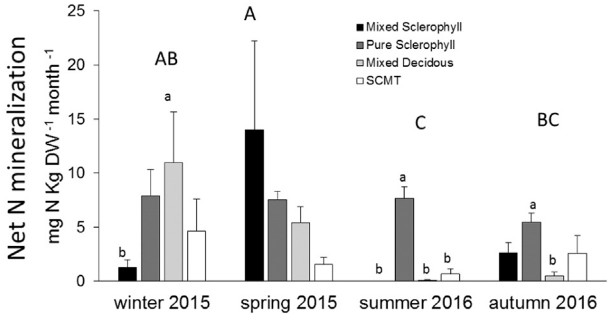

Fig. 4. Field measurements of net N mineralization rates in the surface mineral soil of the sclerophyll forest-type: Mixed sclerophyll, Pure sclerophyll, Mixed deciduous, the Sclerophyll stand near the copper mine tailing (SCMT). Columns represent mean values for each forest stand (n = 6 ± SE). Different capital letters indicate significant differences among seasons, according to Tukey-test. Different lowercase letters indicate significant differences among sites, according to Tukey-test.

soil nitrate contents were found during summer (by 27%) compared to winter (Fig. S1) and the soil nitrate concentrations were about twice higher in the SCMT stand than in all the other less disturbed forest stands (Fig. S1).

Mean annual net N mineralization rates in soils did not differ significantly among the four sclerophyll stands (Table 1S) and >70% of the mean annual rates of net N mineralization originated from nitrification across stands (data not shown).

3.4. Denitrification

Denitrification rates measured in surface mineral soil of these sclerophyll stands also presented significant seasonal and site effects, but there was no effect due to the water addition treatment (Table 5). The highest denitrification rates (up to 70%) were measured during the drier summer period (Fig. 5). The pure sclerophyll stand had significantly (72%) higher denitrification rates than mixed sclerophyll stand (Fig. 5). Significant season*site effects (Table 5) indicate that during summer the mixed deciduous stand presented 85% lower rates of denitrification than both mixed sclerophyll and the pure sclerophyll stands (Fig. 5).

When comparing mean annual denitrification rates in soils the pure sclerophyll stand presented significantly higher rates than all other stands (Table 1S).

3.5. Chemistry of mineral soil, litter and foliage

Bulk density of the mineral surface soils was significantly higher in the disturbed site of SCMT stand (Table 6). The mineral soils of the SCMT presented significantly lower water content, soil reaction, and N/P ratios, but higher soil available N than both the mixed sclerophyll and the pure sclerophyll stands (Table 6). Total soil C, N and P contents were similar in all four stands, however, the disturbed SCMT stand presented significantly lower C/N ratio than all other stands (Table 6). Available P was lower in the SCMT stand than in the pure sclerophyll stand.

Table 5
Two factor ANOVAs repeated measures (season) for denitrification rates in surface mineral soils of the four sclerophyll stands, considering a water addition treatment.

Effect	SS	df	F	P
Site	142.6	3	16.14	<0.001
Treatment	9.5	1	3.24	0.079
Site*treatment	23.2	3	2.63	0.063
Season	114.3	3	14.74	<0.001
Season*site	140.7	9	6.05	<0.001
Season*treatment	22.3	3	2.88	0.039
Season*treatment* site	38.9	9	1.67	0.103

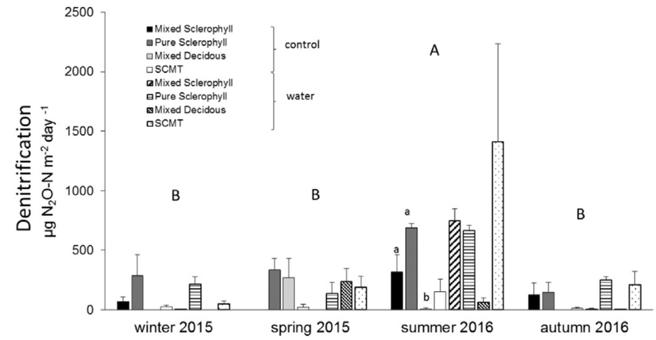

Fig. 5. Field denitrification rates measured in surface mineral soils of the sclerophyll forest-type: Mixed sclerophyll, Pure sclerophyll, Mixed deciduous and the Sclerophyll stand near the copper mine tailing (SCMT). Measurements were made during four seasons with a control (plain columns) and water added (stripped columns) treatment. Columns represent mean values for each forest stand (n = 6 ± SE). Different capital letters indicate significant differences among seasons, according to Tukey-test. Different lowercase letters indicate significant differences among sites, according to Tukey-test. nd = non-detectable.

In the litter layer, mean annual water content was significantly higher in the mixed sclerophyll stand than in all other stands (Table 6). No significant differences were observed in the chemical variables of litter except for a relatively lower C/N ratio in the mixed sclerophyll stand than in all other stands.

In green leaves, the total N concentration of the two co-dominant evergreen tree species in both the pure sclerophyll and SCMT stands, *Lithraea caustica* and *Peumus boldus*, we observed a significant seasonal effect (Table 3S), with about twice higher foliage N contents during spring than in autumn (Fig. 2S). However, there were no site or species effects (Table 3S).

4. Discussion

4.1. Seasonal limitation of microbial N transformations

Climatic seasonality in this sclerophyll-forest type, with a prolonged dry season, strongly influenced microbial N transformations in litter and surface mineral soil in the Mediterranean climatic region of central Chile. DA measured in litter and surface soils, as well as soil net N mineralization rates measured in the field were greatly depressed during summer-autumn compared to winter and spring. In the forests studied, increases of DA in winter and spring coincide with higher rates of net N mineralization. This result provides strong evidence of a temporal coupling between new N inputs to the ecosystem via BNF and internal N recycling via net N mineralization. Confirming the observation that the seasonal summer drought in the four stands of the sclerophyll forest is a limiting factor for free-living DA, experimental water addition to forest soils stimulated DA both in litter during autumn, when water content was <30%, and in surface mineral soils during summer when water content was <10%. However, the lack of response in free-living DA under water treatment during the summer-autumn drought of some sclerophyll stands is suggesting that other factors such as carbon availability may be limiting DA as well (Pérez et al., 2017).

In the Nature Reserve (RCL), containing remnants of evergreen sclerophyll forest-type studied here, Bown et al. (2014) reported that the lowest soil respiration rates occurred during the dry summer. The present study supplements this information by reporting a depressed metabolic activity for both autotrophic and heterotrophic bacteria that fix and process N in the soil solution, making it available for plant uptake. In southern temperate *Nothofagus*-forests farther south, still with strong Mediterranean-climate influence, free-living DA was relatively higher during the austral wet season, both in winter and spring (Pérez et al., 2004, 2009, 2010a, 2010b), and the estimated BNF rates in the litter layer of these wet forests were about one order of magnitude higher

Table 6
Mean annual values of variables in surface soils and litter in four stands of sclerophyll forest in central Chile. Means ± SE ($n = 12$–24). The different letters refer to significant differences among forest stands according to either Tukey or Multiple Comparisons tests.

	Mixed sclerophyll	Pure sclerophyll	Mixed deciduous	SCMT
Surface mineral soil				
Bulk density (g cm^{-3})	0.47 ± 0.11b	0.38 ± 0.04b	0.37 ± 0.05b	0.74 ± 0.07a
Water content (%)	15.93 ± 2.39a	13.71 ± 1.81a	10.81 ± 2.29ab	4.81 ± 1.03b
pH	6.78 ± 0.09a	6.66 ± 0.05a	6.35 ± 0.05ab	4.57 ± 0.14b
N_a (mg kg^{-1})	12.80 ± 1.16b	12.26 ± 0.93b	18.09 ± 1.63ab	25.44 ± 3.01a
% C	7.68 ± 2.12	11.28 ± 1.89	10.12 ± 1.98	2.85 ± 0.47
% N	0.46 ± 0.11	0.65 ± 0.08	0.55 ± 0.10	0.23 ± 0.03
% P	0.021 ± 0.004	0.035 ± 0.003	0.018 ± 0.002	0.018 ± 0.001
C/N	15.27 ± 0.85a	16.85 ± 0.6a	18.26 ± 0.75a	11.41 ± 0.93b
N/P	28.0 ± 7.67b	19.69 ± 2.31b	29.6 ± 3.51ab	12.95 ± 0.97a
P_a (mg kg^{-1})	50.01 ± 12.45ab	33.20 ± 3.51a	19.64 ± 2.43ab	9.86 ± 1.23b
Base cations (cmol kg^{-1})	17.74 ± 1.98	18.82 ± 1.67	14.49 ± 1.43	11.35 ± 2.28
Litter				
Water content (%)	31.14 ± 2.2a	23.68 ± 0.86bc	26.67 ± 1.87b	21.33 ± 0.77c
% N	1.13 ± 0.06	0.96 ± 0.04	0.96 ± 0.07	0.80 ± 0.06
% P	0.031 ± 0.002	0.023 ± 0.002	0.033 ± 0.003	0.023 ± 0.002
C/N	42.67 ± 1.9b	53.14 ± 2.3a	52.34 ± 2.96a	57.52 ± 3.28a
N/P	45.36 ± 5.87	45.35 ± 2.73	42.75 ± 6.28	36.81 ± 1.93

than in the four stands of sclerophyll forest-type reported here (Table 1S).

In Spanish Mediterranean-type shrublands, peak net N mineralization in soils also occurred during winter, with the highest rates of ammonification recorded during spring (Ochoa-Hueso et al., 2014). Similar seasonal differences were observed, in the chaparral vegetation of southern California, where microbial biomass N was maximal during winter, a difference strongly driven by the higher soil moisture availability (Vourlitis et al., 2009). Accordingly, strong limitation of microbial N transformations by soil water availability are consistently observed in Mediterranean-type ecosystems of the world. Moreover, a comparative study of global dryland ecosystems documented that across many sites a decline in total soil N pools was correlated with an increase in the aridity index (Delgado-Baquerizo et al., 2013, 2016). We infer from this information that a regional trend towards more prolonged and intense droughts, which is predicted for the Mediterranean region of central Chile over the coming decades, should cause significant decreases in soil N pools, net N mineralization processes in soil, and associated plant productivity. This decrease will not be compensated by downregulation, because of concomitant water limitation of free-living BNF.

Contrary to our expectations and to our findings regarding the patterns of activity of diazotrophic and N-mineralizing bacteria, we measured the highest gaseous N losses via denitrification during the dry summer season in the sclerophyll forest-type. Experimental water addition in the dry or wet season, at the level applied in the present study (final mean water content ca. 25%), produced no change in the denitrification rates measured in the field, regardless of the site. A similar lack of response of denitrification rates to the experimental addition of water (when soil water content was <2%) has been reported for Italian Mediterranean-type shrublands (Castaldi and Aragosa, 2002). These authors attributed the high rates of denitrification observed in the field during the dry period to denitrifier stabilization in soil colloids, which allowed the bacteria to stay active even during the unfavorable season, without suffering from water stress. Moreover, a particularly broad genetic variability for nir-S genes, a functional marker of denitrifier bacteria, has been reported in Mediterranean-climate shrublands of central Chile, when compared to other arid or semiarid ecosystems (Orlando et al., 2012). Therefore, a variety of different functional types of denitrifying bacteria could be present in Chilean dryland soils, which remain active in spite of the long summer drought, thus enhancing gaseous N losses from ecosystems. Supporting this interpretation, throughfall exclusion experiments in tropical rain forests have reported enhanced N_2O emissions under low soil moisture level (Davidson et al., 2008). In the Mediterranean-climate sclerophyll stands included in the present study, higher soil nitrate contents corresponding with the period of summer-drought and the plant dormant season, may be limiting denitrifier metabolic activity, therefore potentially stimulating N_2O emissions from soils. Accordingly, seasonal studies in southern temperate wet forest soils, have documented an increase in denitrification rates under experimental addition of both nitrate and labile carbon (Pérez et al., 2010a, 2010b).

The product ratio of denitrification $N_20/(N_20 + N_2)$ remains open to question. This ratio tends to decline when soil carbon and water availability increase, but it increases as soil nitrate content grows (Weier et al., 1993; Ciarlo et al., 2008; Senbayram et al., 2012). Accordingly, during prolonged drought in Mediterranean-climate ecosystems, diminished water supply and probably lower labile carbon concentration, resulting from lower decomposition, coincide with high soil nitrate content, thereby enhancing the ratio $N_2O/(N_2O + N_2)$.

The comparison of soil and litter chemical properties, and microbial N transformations in soils and litter across stands studied here, shows that less disturbed sclerophyll forest stands were roughly similar to one another. The most significant differences were found in the analysis of soils from the SCMT stand. The markedly different acid pH of the soils of the SCMT stand may be attributed to the high concentration of cations such as H^+, Fe^{2+} and Al^{3+}, plus the concomitant addition of sulfuric acid to the soil solution derived from the heavy sulfate deposition in the tailing. Addition of toxic elements to the soils may be caused by temporal overflows of the tailing and subsequent sedimentation on the forest floor. In the field, we had visual evidence of such overflows because of the greyish color of sediments, and also because of the higher bulk density and lower water content of the surface mineral soils of the SCMT stand compared to floristically similar but less disturbed sclerophyll forests. Such differences, however, did not translate into correspondingly significant changes in N transformations mediated by soil microbes, except for the lower DA measured in the litter layer of the SCMT stand when compared to the other less disturbed pure and mixed sclerophyll stands. Our results suggest that top soil diazotrophic bacteria are probably more sensitive than N mineralizers and denitrifiers to changes in soil chemical properties, such bulk density, pH and water content, which are subjected to the influence of mine tailings in the Mediterranean region of central Chile.

4.2. Coupling of microbial N transformation in Chilean sclerophyll forest-type

In soils of Mediterranean-type ecosystems of California and Spain, reported total N ranged from 0.04–0.4% ($n = 15$ sites; Stock and

Verboom, 2012). In the four sclerophyll stands included in the present study, soil total N concentrations ranged from 0.23 to 0.65% on average, corroborating previous evidence for a better soil N status in Chilean Mediterranean ecosystems, when compared to other Mediterranean-type ecosystems, despite greater N deposition in northern hemisphere sites (Fenn et al., 2003). From these data, the following paradox emerges. The Chilean Mediterranean-type region, characterized by receiving lower bulk N deposition from atmospheric sources (Vet et al., 2014) than other Mediterranean-type ecosystems, presents however higher soil N content, contrasting with the California chaparral and other sites subjected to a higher atmospheric N deposition (Fenn et al., 2003). We argue that this paradox could be resolved by considering the following features of the N cycle in California chaparral and central Chilean forests: The decoupling of high atmospheric N inputs via dry deposition in the summer months from the winter rain and the growing season in the seasonally dry chaparral vegetation has been the main hypothesis to explain why N limitation persists in high deposition areas (Homyak et al., 2014). In central Chile, on the other hand, despite receiving lower atmospheric inputs of industrial N over the year, significant N inputs to the ecosystem via BNF in winter-spring coincide with the rainy season, and are also coupled with internal pulses of N due to net mineralization in the same seasons. Plant growth and active N uptake in the Mediterranean-type vegetation of central Chile occurs during spring or even during winter for some species (Miller, 1981), which is supported by our data showing higher N content in fresh leaves during spring growing season than during the dry autumn and plant dormant season. Consequently, biologically regulated coupling of inputs via BNF, net N mineralization and plant uptake, minimize potential hydrological losses from the ecosystem, which could explain the long-term N enrichment of soils in Chilean Mediterranean-climate sites. The stream flow is variable among seasons, but is greater in late autumn and winter. However, after the first late autumn rains, the stream water draining the forested watershed in the National Reserve studied showed no detectable concentrations of ammonium and nitrate that could originate from leaching from the ecosystem (Pérez et al., unpublished data), suggesting that these forests are characterized by strong N retention due to biotic uptake. Further, high N losses because of denitrification during the dry summer season should favor greater diazotrophic activity during the winter-spring rainy period, which contributes to replenish gaseous N losses that occurred in summer. Accordingly, tight coupling of microbial N transformations with plant uptake during the wetter growing season could explain the remarkable N fertility of Chilean Mediterranean ecosystem soils, despite receiving relatively low N inputs atmospheric deposition from anthropogenic sources. As an alternative explanation, allelochemical inhibition of nitrification seems less plausible to account for the enrichment in total soil N in the sclerophyll forest-type studied, as >70% of the annual net N mineralization was shown to derive from nitrification in all stands.

5. Conclusions

As expected, microbial N transformations in the sclerophyll forest ecosystem of central Chile showed strong seasonality. Summer an early autumn showed the lowest N inputs to the sclerophyll forest ecosystem via BNF and internal recycling from organic matter via net N mineralization, because of the nearly complete absence of rain during these months. Denitrification rates, however, were considerably higher during the summer dry period than in winter and spring. We propose that strong coupling of biologically driven N fluxes, BNF and internal recycling via N mineralization, with plant uptake during the growing season could explain the relatively higher soil N contents in Chilean sclerophyll forests than in other Mediterranean ecosystems of the northern hemisphere, despite lower atmospheric N deposition. The present evidence suggests that climate model predictions of more extended seasonal droughts in this Mediterranean-type ecosystem could drastically reduce BNF rates and net N mineralization, while in turn enhancing denitrification rates, which is partially controlled by nitrate availability for denitrifiers rather than by water supply. Accordingly, predicted trends towards reduced moisture levels, in the form of mega-droughts, due to climate change may increase the probability of accelerated N depletion in Mediterranean-climate ecosystems of central Chile and elsewhere.

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Appendix A. Supplementary data

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References

Armesto, J.J., Arroyo, M.T.K., Hinojosa, L.F., 2007. The Mediterranean environment of central Chile. In: Veblen, T.T., Young, K.R., Orme, A.R. (Eds.), The Physical Geography of South America. Oxford University Press, New York, pp. 184–199.

Boiser, J.P., Rondanelli, R., Garreaud, R.D., Muñoz, F., 2016. Anthropogenic and natural contributions to the Southeast Pacific precipitation decline and recent megadrought in central Chile. Geophys. Res. Lett. 43. https://doi.org/10.1002/2015GL067265.

Bown, H.E., Fuentes, J.P., Pérez-Quezada, J.F., Frank, N., 2014. Soil respiration across a disturbance gradient in sclerophyllous ecosystems in Central Chile. Cienc. Investig. Agrar. 41, 89–106.

Castaldi, S., Aragosa, D., 2002. Factors influencing nitrification and denitrification variability in a natural and fire-disturbed Mediterranean shrubland. Biol. Fertil. Soils 36, 418–425.

Chapin, F.S., Matson, P., Mooney, H., 2002. Principles of Terrestrial Ecosystem Ecology. Springer.

Ciarlo, E., Conti, M., Bartoloni, N., Rubio, G., 2008. Soil N_2O emissions and $N_2O/(N_2O+N_2)$ ratio as affected by different fertilization practices and soil moisture. Biol. Fertil. Soils 44, 991–995.

CR2, 2015. La megasequía 2010–2015: Una lección para el futuro. Centro de Ciencia del Clima y la Resiliencia (CR)2. www.cr2.cl/megasequia.

Davidson, E.A., Nepstad, D.C., Ishida, F.Y., Brando, P.M., 2008. Effects of experimental drought and recovery on soil emissions of carbon dioxide, methane, nitrous oxide, and nitric oxide in a moist tropical forests. Glob. Chang. Biol. 14, 2582–2590.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Bowker, M.A., Wallenstein, M.D., Quero, J.L., Ochoa, V., Gozalo, V., García-Gómez, M., Solivers, S., et al., 2013. Decoupling of soil nutrients cycles as a function of aridity in global drylands. Nature 502, 672–676.

Delgado-Baquerizo, M., Maestre, F.T., Gallardo, A., Eldridge, D.J., Solivers, S., Bowker, M.A., Prado-Comesaña, A., Gaitán, J., Quero, J.L., Ochoa, V., et al., 2016. Human impacts and aridity differentially alter soil N availability in drylands worldwide. Glob. Ecol. Biogeogr. 25, 36–45.

DeLuca, T.H., Zackrisson, O., Nilsson, M.C., Sellstedt, A., 2002. Quantifying nitrogen-fixation in feather moss carpets of boreal forests. Nature 419, 917–919.

Eno, C.F., 1960. Nitrate production in the field by incubating the soil in polyethylene bags. Soil Sci. Soc. Am. Proc. 24, 277–299.

FAO-UNESCO, 1987. Soils of the World, Food and Agriculture Organization and United Nations Educational Scientific and Cultural Organization. Elsevier Science Publishing Co. Inc., New York.

Fenn, M.E., Baron, J.S., Allen, E.B., Rueth, H.M., Nydick, K.R., Geiser, L., Bowman, W.D., Sickman, J.O., Meixner, T., Johnson, D.W., Neitlich, 2003. Ecological effects of nitrogen deposition in the western United States. Bioscience 53, 404–420.

Groffman, P.M., Holland, E.A., Myrold, D.D., Robertson, G.P., Zou, X., 1999. Denitrification. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 241–257.

Homyak, P.M., Sickman, J.O., Miller, A.E., Melack, J.M., Meixner, T., Schimel, J.P., 2014. Assessing nitrogen-saturation in a seasonally dry chaparral watershed: limitation of traditional indicators of N-saturation. Ecosystems 17, 1286–1305.

Intergovernmental Panel on Climate Change (IPCC), 2013. Summary for policymakers. In: Stocker, et al. (Eds.), Climate Change 2013. The Physical Science Basis. Cambridge Univeristy Press, Cambridge.

Liu, S., Lin, F., Wu, S., Ji, Ch., Sun, Y., Jin, Y., Li, S., Li, Z., Zou, J., 2017. A meta-analysis of fertilizer-induced soil NO and combined $NO+N_2O$ emissions. Glob. Chang. Biol. 23, 2520–2532.

Miller, P.C., 1981. Similarities and limitation of resource utilization in Mediterranean type ecosystems. In: Miller, P.C. (Ed.), Resource Use by Chaparral and Matorral. Springer-Verlag, New York, Inc., pp. 369–407.

Myrold, D.D., Ruess, R.R., Klug, M.J., 1999. Dinitrogen fixation. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 241–257.

Ochoa-Hueso, R., Bell, M.D., Manrique, E., 2014. Impacts of increased nitrogen deposition and altered precipitation regimes on soil fertility and functioning in semiarid Mediterranean shrublands. J. Arid Environ. 104, 106–115.

Orlando, J., Carú, M., Pommerenke, B., Braker, G., 2012. Diversity and activity of denitrifiers of Chilean arid soil ecosystems. Front. Microbiol. 3:1–9. https://doi.org/10.3389/fmicb.2012.00101.

Pérez, C.A., Hedin, L.O., Armesto, J.J., 1998. Nitrogen mineralization in two unpolluted old-growth forests of contrasting biodiversity and dynamics. Ecosystems 1, 361–373.

Pérez, C.A., Carmona, M.R., Armesto, J.J., 2003. Non-symbiotic nitrogen fixation, net nitrogen mineralization and denitrification in evergreen forests of Chiloé Island, Chile: a comparison with other temperate forests. Gayana Bot. 60, 25–33.

Pérez, C.A., Carmona, M.R., Aravena, J.C., Armesto, J.J., 2004. Successional changes in soil nitrogen availability, non-symbiotic nitrogen fixation and carbon/nitrogen ratios in southern Chilean forest ecosystems. Oecologia 140, 617–625.

Pérez, C.A., Carmona, M.R., Fariña, J.M., Armesto, J.J., 2009. Selective logging of lowland evergreen rainforests in Chiloé Island, Chile: effects of changing tree species composition on soil nitrogen transformations. For. Ecol. Manag. 258, 1660–1668.

Pérez, C.A., Carmona, M.R., Fariña, J.M., Armesto, J.J., 2010a. Effects of nitrate and labile carbon on denitrification of southern temperate forest soils. Chil. J. Agr. Res. 70, 251–258.

Pérez, C.A., Carmona, M.R., Armesto, J.J., 2010b. Non-symbiotic nitrogen fixation during leaf litter decomposition in an old-growth temperate rain forest of Chiloé Island, southern Chile: effects of single versus mixed species litter. Austral Ecol. 35, 148–156.

Pérez, C.A., Thomas, F.M., Silva, W.A., Aguilera, R., Armesto, J.J., 2017. Biological nitrogen fixation in a post-volcanic chronosequence from south-central Chile. Biogeochemistry 132, 23–36.

Robertson, G.P., Sollins, P., Ellis, B., Lajtha, K., 1999. Exchangeable ions, pH, and cation exchange capacity. In: Robertson, G.P., Coleman, D.C., Bledsoe, C.S., Sollins, P. (Eds.), Standard Soil Methods for Long Term Ecological Research. Oxford University Press, New York, pp. 241–257.

Rousk, K., Sorensen, P.L., Lett, S., Michelsen, A., 2015. Across-habitat comparison of diazotrophic activity in the subartic. Microb. Ecol. 69, 778–787.

Rousk, K., Sorensen, P.L., Michelsen, A., 2017. Nitrogen fixation in the high artic: a source of “new” nitrogen? Biogeochemistry 136, 213–222.

Rundel, P.W., 1982. Nitrogen utilization efficiencies in Mediterranean-climate shrubs of California and Chile. Oecologia 55, 409–4103.

Sardans, J., Peñuelas, J., 2013. Plant-soil interactions in Mediterranean forest and shrublands: impacts of climate change. Plant Soil 365, 1–33.

Senbayram, M., Chen, R., Budai, A., Bakken, L., Dittert, K., 2012. N_2O emission and the $N_2O/(N_2O + N_2)$ product ratio of denitrification as controlled by available carbon substrates and nitrate concentrations. Agric. Ecosyst. Environ. 147, 4–12.

Shaver, G.R., 1983. Mineral nutrient and nonstructural carbon pools in shrubs from Mediterranean-type ecosystems of California and Chile. In: Kruger, F.J., et al. (Eds.), Mediterranean Type Ecosystems. Springer-Verlag, Berlin, pp. 286–298.

Steubing, L., Fangmeier, A., 1992. Pflanzenökologisches Praktikum. Ulmer, Stuttgart.

Stock, W.D., Verboom, G.A., 2012. Phylogenetic ecology of foliar N and P concentrations and N:P ratios across Mediterranean-type ecosystems. Glob. Ecol. Biogeogr. 21, 1147–1156.

Vet, R., Artz, R.A., Carou, S., Shaw, M., Ro, C.U., Aas, W., Baker, A., Bowersox, V.C., Dentener, F., Galy-Lacaux, C., et al., 2014. A global assessment of precipitation chemistry and deposition of sulfur, nitrogen, sea salt, base cations, organic acids, acidity and pH, and phosphorus. Atmos. Environ. 93, 3–100.

Vitousek, P.M., 1994. Beyond global warming: ecology and global change. Ecology 75, 1861–1876.

Vourlitis, G.L., Pasquini, S.C., Mustard, R., 2009. Effects of dry-season N input on the productivity and N storage of Mediterranean-type shrublands. Ecosystems 12, 473–488.

Walter, H.M., Keeney, D.R., Fillery, I.R., 1978. Inhibition of nitrification by acetylene. Soil Sci. Soc. Am. 43, 195–196.

Weier, K.L., Doran, J.W., Power, J.F., Walters, D.T., 1993. Denitrification and dinitrogen/nitrous oxide ratio as affected by soil water, available carbon, and nitrate. Soil Sci. Soc. Am. J. 57, 66–72.